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Extraction and characterization of gallic acid derivatives from *Pouteria campechiana* for topical anti-ageing nanoemulsion

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ABSTRACT

In view of the adverse effects associated with the chemical route for producing cosmeceutical products and anti-ageing creams, an alternative naturally-sourced nanoformulated anti-ageing cream was suggested. In this study, the extracts of *Pouteria campechiana* (PC) pulp, high in polyphenols was extracted using ultrasonic-assisted extraction and anti-oxidant activities as well as characteristics of the PC crude extract were assessed. The PC pulp extract was subsequently formulated into oil-in-water based nanoemulsion using high speed homogenization. The physicochemical properties and stability of the PC nanoemulsion remained favorable for up to 45 days. For minimizing the negative impact of chemically formulated creams on the human skin, the PC extract nanoemulsion may prospectively be safer and suitable for the human skin.

Keywords: Pouetria campechiana, oil-in-water nanoemulsion, physico-chemical properties.

1. INTRODUCTION

Ageing is a natural phenomenon that transpires to every growing human being which involves a complex biological progression where it is prejudiced by combination of endogenous or intrinsic (genetic, cellular metabolism, hormone and metabolic processes) and exogenous or extrinsic (chronic light exposure, chemicals, toxin) factors [1]. Hence, many consumers have since turned to various commercial over the counter cosmeceutical anti-ageing creams or serums which could cause carcinogenic, possibly causing breast cancer in women, allergic reactions due to preservative [2].

In view of undesirable effects of synthetic ingredients in cosmeceutical products, the search for safer naturally-sourced alternatives as bioactive ingredients in cosmeticeutical products showing good anti-ageing properties, warrants special commercial consideration. Herein, the study highlights a new possible source of anti-ageing compound from a relatively unknown fruit called *Pouteria campechiana* (PC). PC or the 'yolk-fruit' belongs to the Sapotaceae family [3], also called the canistel in English. The seeds and leaves of PC is also reportedly used as remedy for ulcers and inflammatory [4]. The potential of the PC fruit lies in its naturally high content of polyphenols that may be a good source of active ingredient in anti-ageing formulation, the compounds in mind is specifically gallic acid. Moreover, the potential use of Pouteria species for used in anti-ageing justified by its biological activities such as anti-inflammatory, antibacterial [5]

This research will emphasize on synthesizing and characterizing PC pulp into oil-in-water based nanoemulsion which be a better option to maximize the transdermal delivery of the anti-ageing. In this case, resulting PC crude extract will formulated to determine the stability and physico-chemical properties. The particle size and polydispersity index (PDI) of PC nano-formulated are an important parameter in indicating the morphology and assembly of the PC pulp in cosmeceutical products. Application of PC pulp in cosmeceutical can be achieved if the the pH value of nanoemulsion are within the range 4-7.

2. EXPERIMENTAL

The experiment was divided into two main stages. The first stage was focused on the preparation on PC crude extract based on ultrasound-assisted extraction where solution of 60% and 70% (v/v) of ethanol:water was used. The mixture was homogenized using ultrasonic cleaning bath and centrifuged. The extract was filtered and stored in refrigerator. Then, anti-oxidant as well as characteristics of PC crude extract were assessed. After extraction, the second stage was to prepare nanoemulsion of PC pulp extract using high speed homogenization method. At this stage, the oil phase that consists of jojoba oil and grape-seed oil mixture, T80:S20 and glycerin while gum arabic and ultrapure water formed the aqueous phase. In addition, the physicochemical properties and stability of produced nanoemulsion were monitored for changes in particle size and polydispersity index (PDI), respectively.

3. RESULTS AND DISCUSSION

3.1. Extraction and Characterization of PC pulp

The percentage yield of 60% and 70 % (v/v) of ethanol:water was 69.10% and 62.85%, respectively which it affirmed the efficacy of the UAE of polyphenol to draw out the polyphenolic compounds trapped the within the cells of the PC pulp as the yield is relatively high as approximately 70%.

Anti-oxidant of PC pulp extract was revealed by the results of TPC and TFC. TPC of the PC pulp was 523.68 mg GAE/100 gm FWB while TFC showed 397.89 mg GAE/100 gm FWB. It is vital to determine the total phenolic content and total flavonoid content since both are one of the important groups of compounds in secondary metabolites plant species which known to be primary antioxidants for terminating free radicals. Besides that, benefits of flavonoids have been linked to its bioactive properties of being anti-oxidants, protection against cardiovascular disease, certain forms of cancer as well as age related degeneration of cell components. In addition, IC_{50} which correlated from the outcome of the DPPH for the PC pulp extract was estimated as 0.7982 µg/mL. Herein, lower value of IC_{50} is indicative of a stronger antioxidant activity and vary depending on the content of antioxidants compounds present within the samples.

The characterization of PC extract were assessed using fatty acid composition, proximate analysis and acid value. As for analysis of fatty acid composition, fourteen different types of fatty acids were identified in the PC pulp extract as shown in Table 3.1. The predominant acids in descending order were found to be palmitic acid, 16:0 (24.5%), oleic acid, 18:1 (19.1%), myristic acid, 14:0 (16.1%) and linolenic acid, 18:2 (14.1%). Crucially, the high value of myristic acid in the PC pulp extract could be an advantage in terms of cosmeceutical application as the fatty acid has been known for its antimicrobial activity against several bacteria and fungi. The naturally high concentration of myristic acid in topical creams can help prevent acne on the human face [6]. Likewise, the high content of palmitic acid in the PC pulp extract is also beneficial from the perspective of formulating creams to preserve the wellness of the human skin. Crucially, palmitic acid has been known for its skin permeation-enhancing effect [7]. The study is particularly interested in the relatively high presence of linolenic acid in the PC pulp extract. Such fatty acid has been described useful in reducing the sunburn effects and accelerating the healing process of the human skin. The linoleic acid also beneficially curbs the outbreaks of eczema and is the least comedogenic on the skin [8]. Such great properties of the aforementioned fatty acids found abundant in the PC pulp extract favourably infer that the suitability of the PC extract as an active component for developing nanoemulsions to preserve the wellness of the human skin, hence slow down the aging process of the skin.

Other than that, proximate composition in Table 3.2 showed that pulp of the PC is rich in carbohydrates (84.9 %), consistent with reports indicating the fruit being a good source of food and storage of energy while moisture content of the PC pulp was quite low (8.4 %) which suggest the pulp of the PC can be sufficiently dehydrated and would support a longer shelf life of the product, especially when the components of the PC pulp extract are incorporated as the active component in the anti-ageing nanoemulsion.

Table 3.1: Composition of fatty acids in the PC pulp extract

Name	Fatty Acids	Percentage (%)
Caproic acid	C6:0	0.1
Caprylic acid	C8:0	3.1
Capric acid	C10:0	1.2
Lauric acid	C12:0	3.9
Myristic acid	C14:0	16.1
Palmitic acid	C16:0	24.5
Palmitoleic acid	C16:1	4.4
Stearic acid	C18:0	3.3
Oleic acid	C18:1 cis	19.1
	C18:1 trans	< 0.1
Linoleic acid	C18:2 cis	2.4
	C18:2 trans	< 0.1
Linolenic acid	C18:2 cis	14.1
	C18:2 trans	< 0.1
Arachidonic acid	C20:4	0.6
Behenic acid	C22:0	< 0.1
Lignoceric acid	C24:0	3.0
-	Others	4.2

Table 3.2: Proximate composition of the PC pulp

Proximate Analysis Results				
No	**Parameters**	**Test Methods**	**Unit**	**Result**
1	Moisture	In-house STP/FL313/002/07 (based on AOAC 934.01)-Moisture (Loss On Drying)	%	8.4
2	Ash	In-house STP/FL313/001/07 (based on AOAC 923.03)-Ash (Direct Method)	%	1.7
3	Protein	In-house STP/FL313/005/07 (based on AOAC 988.05 & 981.10) –Protein (Kjeldahl Method)	%	4.0
4	Fat	In-house STP/FL313/003/07 (based on BS ISO 8262-3:2005)–Fat (Gravimetric Method)	%	1.0
5	*Carbohydrate	In-house STP/FL313/007/07 based on Methods of Analysis for Nutrition Labeling	%	84.9
6	*Energy Value of Food		kcal/ 100g	364.6

The acid value of the PC pulp extract was found to 6.45 mg KOH/g. The high acid value seen here indicates the PC pulp extract should be esterified prior to formulation to reduce the levels of the free fatty acids, and would consequently improve the storage stability, a key aspect to consider before progressing for formulation into topical skin creams. While identification of phenolic compound present in PC extract was done by HPLC analysis. The chromatograms obtained for the

t_R =2.786 min

GA standard, PC pulp extract and GA-spiked PC pulp extract are shown in Figure 3.1. Based on the area below the peak, it was estimated that GA was present in the PC pulp extract at 0.03 % (w/w), based on the percentage recovery.

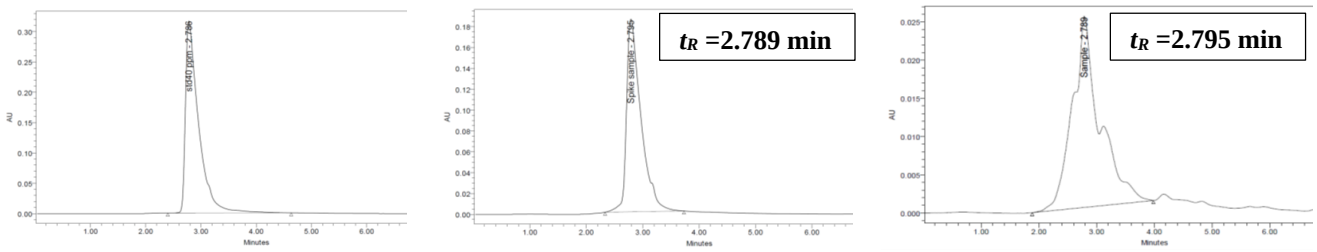

Figure 3.1: HPLC chromatogram at 280 nm of the (a) GA standard, (b) PC pulp extract and (c) spiked PC pulp sample.

3.2 Characterization of nanoemulsion formulated PC pulp extract

In this study, the value of mean particle size and PDI for the PC pulp extract loaded nanoemulsion was found to be 179.40 ± 3.40 nm and 0.262 ± 0.007, respectively. A preferable PDI value should be as low as possible, within the range of 0.1−0.25 to represent a narrow size distribution and is presumptive of an emulsion stable that would remain for a long storage period. The slightly higher PDI value of 0.25 obtained for the prepared PC-pulp extract loaded nanoemulsion is still it is acceptable as it is relatively close to the abovementioned range of PDI values. The particle size of the nanoemulsion were within the required diameter for nanoemulsions between 20−200 nm.

Besides that, no phase separations were observed for the prepared nanoemulsions and the samples remained stable during storage at 25 °C even after 45 days. In contrast, storage at 45 °C saw the nanoemulsion samples remained stable without observable phase separations. However, a creamy phase was formed on the surface of the nanoemulsions at the 45 day of incubation, suggesting the emulsion has begun to destabilize. The results of the storage assessment are shown below in Figure 3.2.

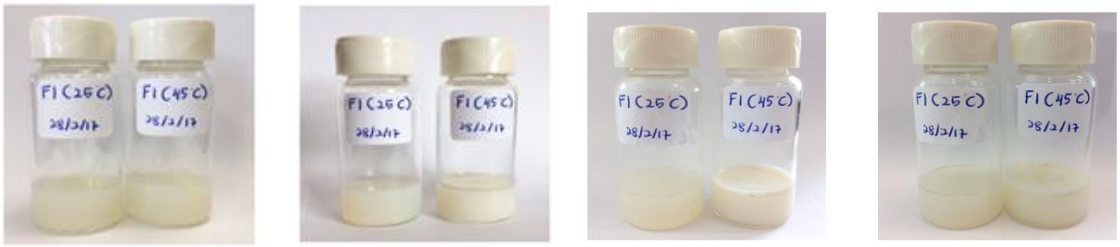

Figure 3.2: Assessment of storage stability at temperatures, 25 °C and 45 °C.

The particle size and polydispersity index (PDI) for 25 °C were also measured during the observation period. These parameters were closely monitored as they were indicators of the stability of the prepared PC pulp extract loaded nanoemulsion. The result was shown below in Figures 3.3 and Figure 3.4.

It evident that the particle size in the prepared nanoemulsions slightly decreased after 30 days of incubation where this phenomenon presumably due to minor occurrences of oxidation process in which the oil soluble molecules were converted into water soluble ones. However, the particle size was observed to marginally increase after 45 days probably due to the effect of Ostwald ripening that tend to lead to coalescence and creaming [9]. Effect of coalescence can be avoided with incorporation of a suitable stabilizer into the formulation. Pertinently, while the particle size of in the nanoemulsions was seen to slightly increase after 45 days, the sized of the nanoparticles were less than 200 nm. The PDI the values after 45 days were also within a satisfactory stable range, 0.1-0.25. This outcome indicates the nanoemulsions were stable throughout the storage period and supported by centrifugal test where the samples remained stable and showed no occurrences of phase separation.

Our findings showed that the pH value of the nanoemulsion for first and at the 45th day were pHs 6.36 and 4.47, respectively. These values seen here was acceptable as the pH of the nanoemulsion remained within the allowable range for formulating topical creams which (pH 4−7), and it will not alter the overall quality of the nanoemulsion and will not irritate the human skin

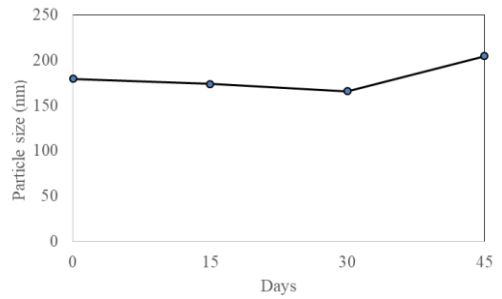

Figure 3.3: Observation of the particle size for up to 45 days

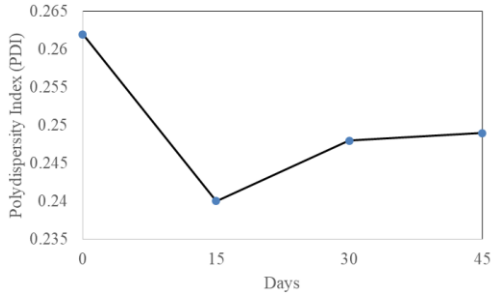

Figure 3.4: Observation on the PDI of the nanoemulsions for up to 45 days

4. CONCLUSION

In this study, the pulp of PC fruit was successfully extracted using ultrasonic assisted extraction method, using the ratio of water:ethanol extraction of 60 % (v/v) that gave a percentage yield of 69.10 % of the PC extract. The PC fruit pulp extract was also successfully characterized and for fatty acid composition, proximate analysis and acid value. The assessments revealed the predominant fatty acid being palmitic acid (16:0), myristic acid (14:0), oleic acid (18:1) and linoenic acid (18:2); with proximate analysis showing carbohydrate (84.9 %), moisture (8.4 %) and protein (4.0 %) as the main component; and the acid value was 6.45 mg KOH/g. All of the above findings revealed the PC pulp components were suitable to be formulated as active ingredients for preparing anti-ageing nanoemulsions. Nanoemulsions contained PC fruit extract was successfully formulated by using combination of high speed homogenization and phase inversion temperature method. The obtained particle size and PDI of the formulated nanoemulsions were well within the required nano-sized regions for nanoformulations, 179.40 ± 3.40 nm and 0.262 ± 0.007 respectively. The creams were also stable in the tests for centrifugation and at different storage conditions of at 25 °C and 45 °C for up to 45 days. pH of the nanoemulsions contained PC fruit extract reported to be suitable to be use on human skin which is in the range of pH 4-7. In general, the study found the protocol to prepare the PC extract formulated nanoemulsions were appropriate to result nanoemulsions that were of good satisfactory condition and had the expected consistency suitable for further encapsulation.

REFERENCES

[1] Ganceviciene. R., Liakou. A.I., Theodoridis. A., Makrantonaki. E. & Zouboulis. C. C. (2012). Skin anti-aging strategies. *Dermato-Endocrinology*. 4(3). pg 308-319.

[2] Chen. Q. (2009). Evaluate the Effectiveness of the Natural Cosmetic Product Compared to Chemical-Based Poducts. *International Journal of Chemistry*. 1(2). pg 57-59.

[3] Hernandez. C. L. C., Villaseñor. I. M., Joseph. E., & Tolliday. N. (2008). Isolation and Evaluation of Antimitotic Activity of Phenolic Compounds from Pouteria Campechiana Baehni. Philippine Journal of Science. 137 (1). pg 1-9.

[4] Pushpakumara D.K.N.G. (2007). Chapter 16: LAVULU. Pouteria campechiana (Kunth) Baehni In: In: Pushpakumara D K N G , Gunasena H P M and Singh V P 2007 (eds) Underutilized fruit trees in Sri Lanka. World Agroforestry Centre, South Asia Office, New Delhi, India. pg 426- 436

[5] Silva. C. A. M., Simeoni. L. A., Silveira. D. (2009). Genus *Pouetria*: Chemistry and Biological Activity. *Brazilian Journal of Pharmacognosy*. 19 (2A). pg 501-509.

[6] Costa. A. Talarico. A. S., Duarte C. O. P., Pereira. C. S., Weimann.E. T. S., Matos. L. S., Coletta. L. C. D., Fidelis. M. C., Tannous. T. S. & Vasconcellos. C. (2013). Evaluation of the Quantitative and Qualitative Alterations in the Fatty Acid Contents of the Sebum of Patients with Inflammatory Acne during Treatment with Systemic Lymecycline and/or Oral Fatty Acid Supplementation. Dermatology Research and Practice. pg 1-14.

[7] Kim. J. W., Lee. B. C., Lee. J. H., Nam. K. C., Ahn. D. U., et al. (2008). Effect of Electro-Beam Irradiation on the Antioxidant Activity of Extracts from Citrus unshiu Pomaces. Radiation Physics and Chemistry. 52. pg 3389-3393.

[8] Zielińska. A. & Nowak. I. (2014). Fatty Acids in Vegetable Oils and Their Importance in Cosmetic Industry. Chemik. 68 (2). pg 103-110.

[9] Bai. L. & McClements. D. J. (2016). Extending Emulsion Functionality: Post-Homogenization Modification of Droplet Properties. Processes. 4(7). pg 1-18.